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Supporting Information

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Supporting Information for

Molecular Photoacoustic Tomography with Colloidal Nanobeacons

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EXPERIMENTAL SECTION

Materials

Unless otherwise listed, all solvents and reagents were purchased from Aldrich Chemical Co. (St. Louis, MO) and used as received. Anhydrous chloroform was purchased from Aldrich Chemical Co. and distilled over calcium hydride prior to use. Biotinylated dipalmitoyl-phosphatidylethanolamine and high purity egg yolk phosphatidylcholine were purchased from Avanti Polar Lipids, Inc. Cholesterol and octylthiol-coated gold nanoparticles were purchased and used as received from Aldrich Chemical Co. (St. Louis, MO). Argon and nitrogen (UHP, 99.99%) were used for storage of materials. The Spectra/Por membrane (Cellulose MWCO: 10 000 Da) used for dialysis was obtained from Spectrum Medical Industries, Inc. (Laguna Hills, CA).

Methods

Typical procedure for preparation of control colloid:

In a typical experimental procedure, the surfactant co-mixture included high purity egg yolk phosphatidylcholine (90 mole%, 558.6 mg), cholesterol (8 mole%, 26.3 mg), and biotinylated-dipalmitoyl phosphatidylethanolamine (2 mole%, 16.2 mg).

The surfactant co-mixture is dissolved in chloroform, filtered and evaporated under reduced pressure, dried in a 40°C vacuum oven overnight, and dispersed into water by probe sonication. This suspension is combined with the almond oil mixture (20% v/v), distilled de-ionized water (77.3% w/v), and glycerin (1.7%, w/v). The mixture is continuously processed thereafter at 20,000 PSI for 4 minutes with an S110 Microfluidics emulsifier (Microfluidics) at 4^{o}C. The nanobeacons are dialyzed against water using a 20,000 Da MWCO cellulose membrane

for a prolonged period of time and then passed through a 0.45 µm Acrodisc Syringe filter. To prevent bacterial growth the nanobeacons are stored under argon atmosphere typically at 4°C.

DLS (D_{av})/nm =154±06 nm; Zeta (ζ)/mV = -26±05 mV.

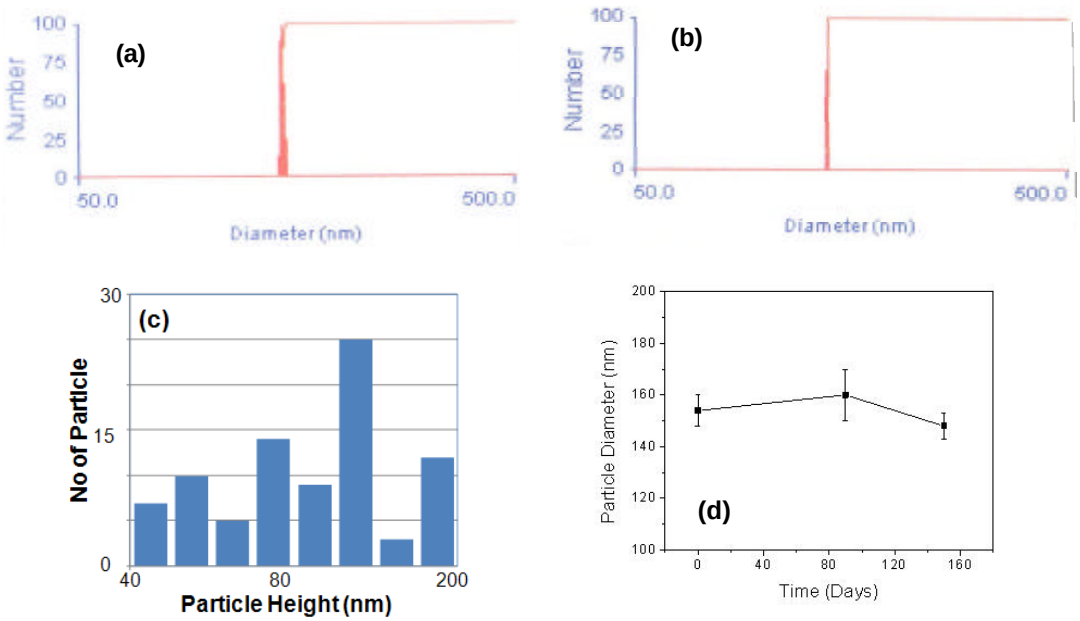

Figure 1. Number-averaged hydrodynamic diameter profiles for (a) GNB and (b) control nanobeacon from dynamic light scattering measurements; (c) distribution of particle height of GNB from AFM analyses; (d) change in particle sizes over time (>150 days stored at 4°C under argon in sealed serum vials).

Measurements

Dynamic light scattering measurements

Instrument and method: Hydrodynamic diameter distribution and distribution averages for the GNB and controls in aqueous solutions were determined by dynamic light scattering.

Hydrodynamic diameters were determined using a Brookhaven Instrument Co. (Holtsville, NY) Model Zeta Plus particle size analyzer. Measurements were made following dialysis (MWCO 10 kDa dialysis tubing, Spectrum Laboratories, Rancho Dominguez, CA) of GNB suspensions into deionized water (0.2 μM). Nanobeacons were dialyzed into water prior to analysis. Scattered light was collected at a fixed angle of 90°. A photomultiplier aperture of 400 mm was used, and the incident laser power was adjusted to obtain a photon counting rate between 200 and 300 kcps. Only measurements for which the measured and calculated baselines of the intensity autocorrelation function agreed to within +0.1% were used to calculate nanoparticle hydrodynamic diameter values. All determinations were made in multiples of five consecutive measurements.

Electrophoretic potential measurements

Instrument and method: Zeta potential (ζ) values for the BiNC were determined with a Brookhaven Instrument Co. (Holtsville, NY) model Zeta Plus zeta potential analyzer. Measurements were made following dialysis (MWCO 10 kDa dialysis tubing, Spectrum Laboratories, Rancho Dominguez, CA) of GNB suspensions into water. Data were acquired in the phase analysis light scattering (PALS) mode following solution equilibration at 25°C. Calculation of ζ from the measured nanoparticle electrophoretic mobility (μ) employed the Smoluchowski equation: $\mu = \varepsilon\zeta/\eta$, where ε and η are the dielectric constant and the absolute viscosity of the medium, respectively. Measurements of ζ were reproducible to within ±4 mV of the mean value given by 16 determinations of 10 data accumulations.

UV-visible Spectroscopy

Instrument and method: Absorption measurements were made with a Shimadzu UV-1601 P/N 206-67001 spectrophotometer using Shimadzu-UV probe 2.21 software.

Transmission Electron Microscopy Measurements

Instrument and method: Glow discharged carbon/formvar coated nickel grids were floated on a drop of sample for 2 mins. Grids were blotted, rinsed quickly in water, and stained in 1% aqueous uranyl acetate for 1 min. Samples were blotted, air dried, and viewed on a Zeiss 902 Electron Microscope, and recorded with Kodak E.M. film. Micrographs were collected at 100,000x magnification. The number-average particle diameter (D_{ah}) values and standard deviations were generated from the analyses of a minimum of 100 particles from three micrographs.

Atomic Force Microscopy Measurements

Instrument and method: A Digital Instruments Dimension 3000 series AFM (calibration date 08/2008) and standard Veeco tapping mode silicon probes w/PtIr coating were used for scanning the samples.

In a typical methodology, aqueous suspensions of GNB samples were dried in a class 10000-clean room on a clean glass slide for 3h. Once dried, samples were placed on the AFM and scanned. Pertinent scanning parameters were as follows: Resonant frequency (probe): 60-80 kHz; Example of tip velocity: (4 um/s for 2um), (15 um/s for 5um), (30 um/s for 10 um). Aspect ratio: 1:1; Lift height: 20 nm; Resolution: 512 samples/line, 256 lines. The average particle

height (H_{av}) values and standard deviations were generated from the analyses of a minimum of 100 particles from three micrographs.

Inductively coupled plasma-optical emission spectroscopy

Instrument and method: After imaging, the gold content of GNB was analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-MS, SOP7040, Rev 9) conducted at the Bodycote, West Coast Analytical Service (WCAS), Santa Fe Springs, CA. Briefly, the samples were analyzed by a Leeman Labs Direct Reading Echelle ICP-MS, or a DRE instrument which was designed to handle sub-ppm to percent level metal concentrations.

In vitro human plasma clot phantoms

Method: In a typical procedure, whole porcine blood was obtained fresh and anticoagulated (9:1, vol/vol) with sterile sodium citrate. Plasma clots were produced by combining plasma and 100 mmol/L calcium chloride (3:1 vol/vol) with 5 U thrombin (Sigma-Aldrich, Inc.) in a (low density polyethylene) tube (~1 cc volume, I. D. ~6 mm). The plasma was allowed to coagulate slowly at room temperature. The clots were incubated individually with 150 µg biotinylated antifibrin monoclonal antibody (NIB5F3)[1] in 10 mL PBS with 1% crystalline BSA (Sigma Chemical Co) for 2 hours. The antibody-treated clots were then incubated with excess avidin (50 µg/mL PBS) for 30 minutes, followed by biotinylated GNB (30 µL/mL PBS) for 30 minutes. The control clots were treated similarly with control nanoparticle (30 µL/mL PBS).

Staining of human plasma clot phantoms

Method: Biebrich Scarlet-Acid Fuchsin Solution was diluted 1:1 in 1XPBS. Plasma clot targeted with GNB and control samples were incubated with 200ul diluted staining solution on the

surface at room temperature for five minutes, then wash with 1XPBS for three times. Keep samples in 1XPBS buffer at refrigerator overnight.

Photoacoustic Tomographic System

Instrument and method (*In vitro* photoacoustic imaging of targeted gold nanobeacons): A curved array photoacoustic tomography system[2] was used for *in vitro* imaging of the targeted plasma clot samples. The light source was same as used before for the PA spectrum study. A uniform illumination area of approximately 20 mm in diameter on the sample surface was produced by diverging the laser beam with a concave lens and homogenizing it by a circular diffuser. The sample was placed at the center (focal point of the curved transducer) and was illuminated orthogonal to the imaging plane of the transducer for maximum uniformity. The transducer consisted of 128 elements arranged along a 90° arc with a 25 mm center of curvature (5 MHz central frequency, BW 80%, custom manufactured Imasonic Inc., Besançon, France).The transducer used piezocomposite technology for high sensitivity and signal-to-noise ratio. Individual elements had an elevation height of 10 mm with an azimuthal pitch of one wavelength (0.308 mm) and kerf of 0.1 mm. Electronic beam formation provided in-plane dynamic focusing. PA signals from each element were amplified with 60-70 dB gain and multiplexed into 16 parallel data acquisition channels. The data was DMA-transferred to RAM and subsequently to disk for post-processing. The acquisition rate was 1 frame/second. Images were reconstructed using a delay-and-sum algorithm.[3]

Photoacoustic breast scanner system

Instrument and method (*In vitro* photoacoustic imaging of targeted gold nanobeacons): A photoacoustic breast scanner system[4] was used for *in vitro* imaging of targeted GNB. The light

source was 532 nm wavelength. A Q-switched Nd:YAG laser with a 10 Hz pulse repetition rate, 5 ns (@532 nm wavelength) laser pulse width, and 400 mJ maximal output energy was the light source. The incident laser fluence on the sample surface was controlled to < 20 mJ/cm^2, conforming to the American National Standards Institute (ANSI) standards.[5] The generated acoustic signal was detected using a 13-mm-diameter active area nonfocused single element transducer operating at a 2.25 MHz central frequency (ISS 2.25 X 0.5 COM, Krautkramer). The signal was first amplified by a low-noise pulse amplifier (5072PR, OlympusNDT), then filtered electronically, and finally recorded using a digital data acquisition card (14 bit Gage Card). As before, images were reconstructed using a delay-and-sum algorithm.[4]

Animal and Drug Information:

Guidelines on the care and the use of laboratory animals at Washington University in St. Louis were followed for all animal experiments. Adult Sprague Dawley rats with various body weights (250 – 350 g) were used for the experiments. Initial anesthetization of the rat was done using a mixture of ketamine (85 mg/kg) and xylazine (15 mg/kg). The hair on the region of interest of the rat was gently removed before imaging, using a commercial hair-removal lotion. Intradermal injection of 0.075 ml of gold nanobeacons (GNB) was performed on a left/right forepaw pad, depending on which side was imaged. PA images were acquired after the administration of GNB. During the image acquisition, anesthesia was maintained using vaporized isoflurane (1 L/min oxygen and 0.75% isoflurane, Euthanex Corp.), and a pulse oximeter (NONIN Medical INC., 8600V) was used to monitor the vitals. If needed, 8 ml of 0.9% saline was administered to the rat for hydration. After image acquisition, the animal was euthanized by pentobarbital overdose. For intravenous injection the doses was 3 ml/kg.

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